further believes that Boss, *et al.* teaches the isolation and monolayer culture of human mesencephalon neural progenitor cells, while Gallyas, *et al.* discloses the characterization of mouse immortalized neuronal cell lines by measuring the concentration of various neurotransmitters. (Office Action, pages 4-5). The Examiner further believes that Weiss *et al.* teaches a method for producing neuronal stem cells using various proliferation-inducing growth factors. (Office Action, page 5). The Examiner believes that it would have been obvious to modify the teachings of Hoshimaru *et al.* and Prasad *et al.* with the substitution of human mesencephalic cells as taught by Boss *et al.* (*Id.*). The Examiner further believes it would be obvious to characterize immortalized human mesencephalic cells as described by Gallyas *et al.* because GABA and dopamine are neurotransmitters of interest. (*Id.*).

Contrary to the Examiner's assertion, Applicants have indeed considered the combined teachings of the cited references. Where Applicants have addressed the references individually, it has been to rebut a point for which the Examiner has specifically cited the reference. Applicants' position has been, and remains, that the Examiner has simply selected convenient aspects of the cited references without fully considering the disparate teachings of that art, teachings that would discourage their combination. Applicants respectfully disagree with the Examiner's assertions and conclusions, and traverse this ground of rejection.

A. <u>The Law of Obviousness</u>

The initial burden is on the Examiner to make out a *prima facie* case of obviousness. MANUAL OF PATENT EXAMINING PROCEDURE (MPEP) § 2142, at 2100-96. To establish a *prima facie* case of obviousness, three criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge available to one of skill in the art to combine the references. Second, there must be a reasonable expectation of success. Finally, the prior art references, when combined, must teach or suggest all of the claim limitations. MPEP § 2142, at 2100-97.

B. <u>The Cited References Cannot Be Combined to Reject the Claimed Invention as Obvious</u>

The combination of references cited by the Examiner fails because one of skill in the art would not combine these references to attain the claimed invention. Neither the cited references nor the art itself suggests combining the references.

Applicants point out that they were the first to produce conditionally-immortalized *human* mesencephalic cells, and to differentiate them into mature mesencephalic neural cells. This satisfied a long-felt need in the art because they would be "valuable research tools," as the Examiner has stated. However, the primary art cited by the Examiner against the present invention dates to more than *two* years prior to Applicants' filing date; the Hoshimaru *et al.* reference published in February 1996 (reporting work that had been done in 1995), and the Boss *et al.* reference claims priority to an application filed in

1989. Moreover, one of the inventors (Dinah Sah) on the instant application, filed in 1998, is one of the authors of the Hoshimaru *et al.* reference. The clear reason for the later filing, therefore, is that the present invention is not, in fact, obvious, and its execution far less straightforward and routine than the Examiner believes it was at the time of filing.

1. There is No Motivation to Combine the Cited References

The Examiner states that it would be obvious for one of skill in the art to substitute the cells of Boss *et al.* into the method of Hoshimaru *et al.* (Office Action, at page 5). However, there is no motivation to combine the cited references. Hoshimaru *et al.* does not teach or suggest the immortalization of human mesencephalon cells; Hoshimaru *et al.* teaches the conditional immortalization of *rat hippocampal* cells. Hoshimaru *et al.* does not teach that the method disclosed therein can be used to conditionally immortalize human mesencephalic cells. Boss *et al.* teaches the production of what are characterized as non-immortalized human mesencephalon progenitor cells. Boss does not suggest the immortalization, conditional or otherwise, of the cells disclosed therein. Moreover, it is well known in the art that different regions of the brain require different culture conditions. Thus, there is no motivation to combine Hoshimaru *et al.* with Boss *et al.*, and the two references cannot be combined to reject the claimed invention as obvious.

Hoshimaru *et al.* teaches the conditional immortalization of *rat* cells with tetracycline-regulated *v-myc*. Prasad *et al.* teaches the conditional immortalization of *rat* cells with SV40 and polyoma virus T antigens. Neither Hoshimaru *et al.* nor Prasad *et al.* teach or suggest the conditional immortalization of *human* mesencephalic cells with these immortalizing agents. Boss *et al.* does not remedy this deficiency, as it teaches only the culture of non-immortalized neuronal progenitor cells. Thus, the three references cannot be combined to reject the claimed invention as obvious.

The Examiner states that Prasad *et al.* "teaches that mesencephalic cell[s] could be genetically manipulated." With respect, Applicants argue that this is irrelevant to the instant invention. Prasad *et al.* does not teach that their methods are applicable to human cells. Rather, Prasad *et al.* very specifically teaches only that the two SV40 constructs used could conditionally immortalize *rat* cells. Because this teaching is limited in scope, it cannot be used to demonstrate a reasonable expectation of success in applying the disclosed methods to *human* cells. The citation of Prasad *et al.* in combination with the remaining references is, therefore, inappropriate.

The Examiner further states that "immortalized human neuronal progenitor cells are valuable research tools . . ." However, the desire for a particular result is not a motivation to combine two references; there has to be some teaching in the references themselves or in the art that the references can *be* combined. There is none in the cited references.

Finally, the Examiner does not take into account the wide variety of methodologies taught by the cited art, as previously pointed out by Applicants in Paper No. 25. A person of skill in the art would not be guided by these various teachings to the practice the methods and cells claimed in the instant application.

2. The Cited References Do Not Provide a Reasonable Expectation of Success in Practicing the Claimed Invention

The Examiner also fails to establish a reasonable expectation of success in practicing the claimed invention through the combinations of cited art. The Examiner states that "one would have a reasonable expectation of success because neural progenitor cells are easy to transfect . . ." (Office Action, at p. 5). In fact, primary human cells are more difficult to infect than rat cells because of the different proteins on the human cells' surface. Moreover, the invention is more than simply transfecting cells; ease of transfection does not mean that a person of skill in the art would have a reasonable expectation of practicing *the invention* as *claimed*. In fact, given the difficulty of establishing immortalized human neuronal cell lines, a person of skill in the art at the time of filing would have viewed any combination of *rat*-related art as worth trying, but not promising success.

The assertion of obviousness made by the Examiner is, therefore, essentially that it would be obvious to *try* substituting the rat cells used in Hoshimaru, *et al.* with human cells. However, "obvious to try" is an improper basis for a §103(a) rejection. *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988). Thus, the invention as embodied in the present invention cannot be rendered obvious by the combination of cited references.

3. The Cited References Do Not Teach Every Limitation of the Claims

The art cited by the Examiner fails to teach every limitation of the claimed invention. Our reasoning is as follows.

The Examiner cites Hoshimaru *et al.* as teaching the use of a factor (forskolin) for differentiation (Office Action, at page 4), in a rejection of at least claims 9, 10, and 25-27. The Examiner, however, misconstrues this reference's actual teaching. At page 1522, Hoshimaru states that *previous studies* had found that "several cytokines, or forskolin or growth factors on specific substrates" were needed for differentiation. In contrast, Hoshimaru *et al.* teaches that "suppression of the *v-myc* production is sufficient to differentiate immortalized neuronal progenitor cells into neurons." (*See* page 1521, right column, heading). Hoshimaru therefore clearly teaches *away* from the use of forskolin, or any other factor, to differentiate progenitor cells expressing an immortalizing gene from a regulatable promoter. The presently claimed cells and the cells obtained by the presently claimed methods express an immortalizing gene from a regulatable promoter. Thus, this reference cannot be combined with the remaining references to reject claims 9, 10 and 25-27 as obvious.

The Examiner also misconstrues the teachings of Weiss *et al.*, which the Examiner cites against claims 1-5, 9, 10 and 25-27. Applicants note that the combination of Hoshimaru *et al.*, Boss *et al.*, and Prasad *et al.* does not teach the combination of EGF, FGF-2 and PDGF in the culture of conditionally-immortalized human mesencephalic progenitor cells, and does not teach the combination of differentiating factors recited in claims 25 and 26. Thus, these missing teachings must be supplied by Weiss *et al.*

At col. 17, lines 1-15, Weiss *et al.* suggests that cells be proliferated in EGF and FGF-2. PDGF is suggested to *influence differentiation* (col. 17, line 12). According to claim 1 of the instant method, differentiation is strictly controlled by the expression of the oncogene. Moreover, Weiss *et al.* teaches that, after culturing in "a proliferation-inducing growth factor," the disclosed stem cells "begin[] to divide, giving rise to a cluster of undifferentiated cells referred to herein as a 'neurosphere'." (col. 17, lines 17-20). These "neurospheres" are obviously not the monolayer taught by the instant disclosure. Weiss therefore teaches that the combination of EGF and FGF-2 with PDGF has a different *purpose* and achieves a different *result* than in the instant invention. Therefore, Weiss *et al.* does not rectify the deficiencies of the remaining cited art, and cannot be combined with them to render the instantly-claimed invention obvious.

Other sections of Weiss cited by the Examiner are irrelevant or do not supply the teachings for which the Examiner cites Weiss *et al.* Col. 22, lines 17-29 teach that various "growth factor products" may be useful in the treatment of CNS disorders. None of the claims of the instant invention are directed to the treatment of CNS disorders. Col. 30, line 17 merely refers to a section heading and provides no useful information. Col. 31, lines 46-64 disclose a list of "biological agents" that may be *tested* to determine their effects on precursor cells (*see* col. 31, lines 29-45). It is clear that the patentee had no idea what the effects of those compounds would be, only that their effects could be tested. Finally, Examples 1-6 teach only the use of EGF in the culture of mouse neural stem cells; Example 7 teaches *differentiation* in EGF-containing medium; and Example 8 teaches the use of CNTF, BDNF or FGF-2 on *differentiation* of the neurospheres.

Therefore, Weiss *et al.* does not teach the culture of conditionally-immortalized human mesencephalon progenitor cells in EGF, FGF-2 and PDGF to produce the cells of the invention, as recited in claim 1. Because Hoshimaru *et al.* teaches only the culture in FGF-2 (see page 1519, left column, second full paragraph), Prasad *et* al. teaches only EGF (*see* page 597, right column, first full paragraph, reference 37), and Boss *et al.* teaches *none* of EGF, FGF-2 and PDGF, the combination of these four references does not teach the use of EGF, FGF-2 and PDGF as recited in claim 1 of the instant invention. Because claims 9, 10 and 25-27 depend upon claim 1, the combination of references likewise cannot be used to reject these claims as obvious. Weiss moreover does not teach the combination of GDNF and CNTF, or GDNF, CNTF, IGF-1 and BDNF in the *differentiation*

of the conditionally-immortalized human mesencephalon progenitor cells, as recited in claims 25 and 26, respectively. Hoshimaru *et al.*, as explained above, does not teach differentiation with forskolin. Because the cited references *in combination* do not teach differentiation with the combination of forskolin, GDNF and CNTF, or forskolin, GDNF, CNTF, IGF-1 and BDNF, the combination of references cannot be used to reject claims 25 and 26 as obvious.

The Examiner, once again, cites Boss *et al.* as teaching "monolayers," citing the abstract and column 11, line 25. Applicants are aware that Boss *et al.* uses the word "monolayer" in the heading of the section that begins at column 11, line 25. However, the Applicants respectfully suggest that the Examiner has ignored *other* statements in Boss *et al.* that indicates that a "monolayer," as that term is used in the art and in the instant claims, is actually not disclosed. Boss *et al.* specifically states that "[g]ross examination of typical neuron progenitor cell 'monolayer' cultures reveals interconnected three-dimensional structures, rather than the usual two-dimensional monolayer observed with most cell lines."[1] Col. 6, ll. 4-7. Thus, rather than a monolayer of cells, Boss *et al.* actually discloses cells in interconnected three-dimensional structures. In contrast, claim 6 of the instant application clearly recites "*adherent* monolayers" - *i.e.*, a two-dimensional layer of cells. The Examiner has still not explained how the clumps of cells disclosed in Boss *et al.* are adherent monolayers. A reference must be cited for what it *fairly* suggests, *In re Burkel*, 201 U.S.P.Q. 67 (C.C.P.A. 1979), and Boss fairly suggests something other than the adherent monolayers recited in claim 1. Boss *et al.* therefore clearly does *not* teach monolayers, as recited in claims 1 and 6, and cannot be combined with Hoshimaru *et al* and/or Prasad *et al* to render these claims obvious.

Claims 13-15, 23 and 24 are also not obvious over the cited art. The Examiner has not made clear which reference is being applied to assert the obviousness of claims to cells, but Applicants will assume it is the Hoshimaru *et al.*, Prasad *et al.* and Boss *et al.* references. Hoshimaru *et al.*, Prasad *et al.* disclose rat cells. Applicants wish to point out that rat cells are compositions of matter that are substantially different from human cells. The two come from a completely different source, have different biochemical markers, and react differently to culture and proliferation conditions. Rat cells are considered substantially different than human cells by those in the art. The Examiner has provided no reference suggesting or teaching what modifications of the rat neuronal cells should be performed to arrive at the claimed human neuronal cells.

The Examiner states that "it would have been obvious . . . to substitute the immortalized rat neuronal progenitor cells as taught by Hoshimaru *et al.* and Prasad *et al.* with human mesencephalon neuron progenitors as taught by Boss *et al.*" (Office Action, at p. 5). This fails to explain how the cell claims of the instant invention are obvious, however,

[1] Indeed, Boss *et al.* here places "monolayer" in quotes to indicate that the cultures are not monolayers as persons of skill in the art recognize them.

because the fact that one cell may substitute for another does *not* mean that the second is obvious in light of the first. Applicants note, too, that the Examiner has not suggested that the cells of Boss *et al.* could be conditionally-immortalized.

Applicants respectfully suggest that the Examiner may have confounded the product disclosed in the references with the process used to make them. For example, the Examiner states that "it would have been obvious . . . to substitute the immortalized rat neuronal progenitor cells as taught by Hoshimaru et al and Prasad et al with human mesencephalon neuron progenitor cells as taught by Boss et al." (Emphasis added.) In essence, the Examiner argues that the method of Hoshimaru makes the claimed conditionally-immortalized progenitor cells, and the resulting differentiated cells, obvious. In making this argument, the Examiner follows essentially the same obviousness analysis disallowed in *In re Deuel*, 51 F.3d 1552 (Fed. Cir. 1995) (method of obtaining a DNA molecule cannot render obvious the DNA molecule itself). Thus, the general method of making immortalized *rat* neuronal progenitor cells taught in Hoshimaru *et al.* cannot render obvious the claimed conditionally-immortalized human neuronal progenitor cell itself.

Thus, the combination of Hoshimaru *et al.*, Prasad *et al.* and Boss *et al.* references do not render the cell of the instant invention, or of claims 6, 13-15, 23 and 24, obvious.

Finally, the Examiner once again rejects claims 7 and 8 in part over Gallyas *et al.*, because the Examiner believes that the reference teaches the characterization of mouse immortalized neuronal cell lines by measuring the concentration of various neurotransmitters such as GABA and dopamine. Gallyas, *et al.* is irrelevant to claim 7 and claim 8 because neither of these claims recites *methods* for identifying GABAergic or dopaminergic neurons; the claims are directed to conditionally immortalized *cells* that can differentiate into neurons that are GABAergic or dopaminergic. Applicants respectfully restate that the Examiner cites Gallyas *et al.* for the wrong proposition; thus the reference cannot be used in combination with any other cited reference to reject claims 7 and 8.

CONCLUSION

Applicants respectfully request entry of the foregoing remarks. No fee is believed due. If a fee is required in connection with this Response, please charge Pennie & Edmonds LLP Deposit Account Number 16-1150 for the appropriate amount.

Respectfully submitted,

Date March 20, 2003

By: Lawrence B. Graham Reg. No. 49,020
Anthony M. Insogna Reg. No. 35,203

Anthony M. Insogna (Reg. No.)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

EXHIBIT A
CLEAN VERSION OF THE CLAIMS THAT WILL BE PENDING UPON ENTRY OF THE PRESENT AMENDMENT
U.S. PATENT APPLICATION SERIAL NO. 09/134,771

1. (Amended three times) A method for producing a conditionally-immortalized human mesencephalon neural progenitor cell, comprising:

(a) plating human mesencephalon cells on a first surface and in first growth medium that permits proliferation;

(b) transfecting said progenitor cells with DNA encoding a selectable marker and an externally regulatable growth-promoting protein; and

(c) selecting an adherent monolayer of the transfected cells on a second surface and in a second serum-free growth medium that permits attachment and proliferation, wherein the second serum-free growth medium comprises FGF-2, EGF and PDGF, and therefrom producing a conditionally-immortalized human mesencephalon cells in which the growth-promoting protein is regulated by an external factor, such that suppression of the growth promoting protein results in differentiation of the cell into a neuron.

2. The method of claim 1 wherein the first and second surfaces are independently selected from the group consisting of substrates comprising one or more of a polyamino acid, fibronectin, laminin or tissue culture plastic.

3. The method of claim 1 wherein the growth-promoting gene is an oncogene.

4. The method of claim 3 wherein the oncogene is v-myc.

5. The method of claim 1 wherein expression of the growth-promoting gene is inhibited by tetracycline.

6. (Twice amended) A conditionally-immortalized human mesencephalon neural progenitor cell capable of differentiation into neurons, wherein the cell is transfected with DNA encoding a growth-promoting protein that is regulated by an external factor, such that suppression of the growth-promoting protein results in differentiation of the cell into a neuron, and wherein the cell is polygonal and grows as an adherent monolayer.

7. A conditionally-immortalized human mesencephalon neural precursor cell according to claim 6, wherein the cell is capable of differentiation into dopaminergic neurons.

8. A conditionally-immortalized human mesencephalon neural precursor cell according to claim 6, wherein the cell is capable of differentiation into GABA-ergic neurons.

9. (Amended) A method for producing a neuron, comprising culturing a cell produced according to claim 1 in the presence of at least one differentiating agent under conditions that inhibit expression of the growth-promoting gene.

10. A method according to claim 9, wherein the cell is cultured in medium comprising tetracycline.

13. A neuron produced according to the method of claim 9.

14. A dopaminergic neuron produced according to the method of claim 9.

15. A GABA-ergic neuron produced according to the method of claim 9.

23. A conditionally-immortalized human mesencephalon neural precursor cell produced according to the method of claim 1.

24. A cell according to claim 23, wherein the cell is present within a clonal cell line.

25. (Amended) The method of claim 9, wherein the differentiating agent comprises the combination of forskolin, GDNF and CNTF.

26. (Amended) The method of claim 9, wherein the differentiating agent comprises the combination of forskolin, GDNF, CNTF, IGF-1 and BDNF.

27. (New) The method of claim 9 wherein said differentiating agent comprises GDNF.